510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY ONLY TEMPLATE

A. 510(k) Number:

k121101

B. Purpose for Submission:

New device

C. Measurand:

Galactose and galactose-1-phosphate

D. Type of Test:

Quantitative, galactose dehydrogenase absorbance method

E. Applicant:

Astoria-Pacific, Inc.

F. Proprietary and Established Names:

Astoria-Pacific SPOTCHECK® Neonatal Total Galactose Microplate Reagent Kit

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
JIA	Class I, exceeds the limitation to exemption in 862.9(c)(2)	21 CFR 862.1310 - Galactose test system	Chemistry

H. Intended Use:

1. Intended use(s):

   The SPOTCHECK Neonatal Total Galactose Microplate Reagent Kit is intended for the quantitative determination of the concentration of Total Galactose (galactose (Gal) + galactose-1-phosphate (Gal-1-P)) in whole blood saturated filter paper disks using a microplate absorbance reader or SPOTCHECK Pro. Measurements of Total Galactose are used primarily in the diagnosis and treatment of the hereditary disease galactosemia. This method is intended for *in vitro* diagnostic use as an aid in neonatal screening for increased concentrations of Total Galactose, and not for monitoring purposes.

2. Indication(s) for use:

   See intended use above.

3. Special conditions for use statement(s):

   For *In Vitro* Diagnostic Use. For prescription use only.

   The device labeling states that specimens producing elevated (presumed positive for galactosemia) require confirmation or follow-up testing according to local, state and federal requirements.

4. Special instrument requirements:

   The SPOTCHECK Neonatal Total Galactose Microplate Reagent Kit is designed for use with a 96-well microplate absorbance reader, equipped with a 600 nm measurement channel and a 750 nm reference channel. In this submission, a TECAN Sunrise microplate reader was used to generate data for the manual method.

   The SPOTCHECK Neonatal Total Galactose Microplate Reagent Kit is also designed to be used with the automated SPOTCHECK Pro™ platform.

I. Device Description:

Each Neonatal Total Galactose (TGAL) kit contains the following components:

Galactose calibrators and reagents are shipped lyophilized. The stock standards and final calibrator concentrations (after preparation followed by spotting onto filter paper) are:

Stock Standard	Final Concentration
0 mL	0 mg/dL
0.5 mL	5 mg/dL
1.0 mL	10 mg/dL
2.0 mL	20 mg/dL
3.0 mL	30 mg/dL
5.0 mL	50 mg/dL

All reconstituted reagents have a volume of 450 mL, and are as follows:

- Extraction solution with trichloroacetic acid (final concentration 0.2 M)
- Enzyme reagent with alkaline phosphatase (final concentration 340 kU/L) and galactose dehydrogenase (final concentration 2.1 kU/L)
- Coenzyme reagent with nicotinamide adenine dinucleotide (final concentration 9.4 mM)
- Color reagent with 1-Methoxy PMS (final concentration 60 μM) and MTT (final concentration 97 μM)

The labeling contains the following precautionary statement: Reagents in this kit contain sodium azide as a preservative. Sodium azide can react with lead and copper plumbing to form explosive metal azides. When disposing of reagents, flush with large

volumes of water to prevent azide build-up. Dispose of all waste in accordance with all local, state and federal regulations.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Accuwell Total Galactose

2. Predicate K number(s):

   k991498

3. Comparison with predicate:

Characteristics	Predicate Device (k991498)	Proposed Device
Similarities		
Intended Use	Is intended to be used for the quantitative measurement of Total Galactose in neonatal dried blood spot samples. Results are used to screen newborns for galactosemia.	Same
Specimen collection, handling and storage	Use standardized blood spot collection cards; follow protocol in *CLSI LA4-A5*	Same
Extraction and incubation	In microplate, on shaker	In microplate, on combination incubator/shaker
Incubation time	30 – 60 minutes	30 minutes
Extraction Solution	3% TCA	Same
Enzyme Reagent	Buffered Alkaline Phosphatase, Galactose Dehydrogenase (and Nicotinamide adenine dinucleotide (NAD) coenzyme)	Buffered Alkaline Phosphatase and Galactose Dehydrogenase
Coenzyme reagent	NAD included in enzyme reagent (see above)	NAD
Specimen	1 x 1/8” dried blood spot (DBS) disks (with second	1 x 1/8” dried blood spot (DBS) disks

	protocol using 2 x 1/8" disks)	
Color reagent	Buffered MTT + Methoxy PMS	Same
Reporting units	mg/dL	Same
Characteristics	**Predicate Device (k991498)**	**Proposed Device**
Differences		
Extraction and incubation temperature	18-25 °C	37 °C
Extraction time	45 – 120 minutes	60 minutes
Absorbance measurement	570 nm (690 nm reference)	600 nm (750 nm reference)
Limit of quantitation	1.5 mg/dL	1.4 mg/dL
Range	1.5 mg/dL – high calibrant (value changes)	1.4 – 50 mg/dL
Calibration	Dried blood spot calibrators	Liquid galactose calibrators
Quality control material	Dried blood spots with low, mid, and high levels of galactose	Not provided with kit

K. Standard/Guidance Document Referenced:

- CLSI document EP5-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline*
- CLSI Guideline EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*
- CLSI Protocol EP7-A2: *Interference Testing in Clinical Chemistry*
- CLSI Protocol EP17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*

L. Test Principle:

Total Galactose is measured colorimetrically following the completion of two enzyme assisted reactions and the color formation reaction. The first reaction entails conversion of Galactose-1-Phosphate (Gal-1-P) to Galactose (Gal), catalyzed by alkaline phosphatase.

Alkaline Phosphatase → Gal-1-P Gal

In the second reaction, Gal is converted to galactonolactone through the galactose dehydrogenase Nicotinamide adenine dinucleotide redox pair (NAD+/NADH)-coupled reaction.

Galactose Dehydrogenase → Gal + NAD+ galactonolactone + NADH

The NADH produced is proportional to the Gal concentration.

The final reaction, catalyzed by 1-methoxy PMS, employs a tetrazolium salt (MTT) and produces a formazan dye that is measured colorimetrically.

1-methoxy PMS → NADH + MTT Colored Formazan + NAD+

The color developed is proportional to the Total Galactose concentration in the sample. A standard curve prepared from a stock Galactose solution is used to quantitate the results.

M. Performance Characteristics:

1. Analytical performance:

   a. Precision/Reproducibility:

   The within-run and total precision of the SPOTCHECK Neonatal Total Galactose Microplate Reagent Kit was evaluated according to the CLSI Guideline EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods* with modifications.

   Evaluation of precision utilized samples that were prepared from whole blood, with hematocrit adjusted to 55%, and spiked with analyte at three different concentrations typical for "normal" (3 mg/mL), "near cutoff" (8 mg/dL) and "galactosemic" (27 mg/dL). Samples were analyzed over five days, one run per day, sixteen replicates of each sample per run.

   To evaluate the imprecision due to multiple calibrations, two calibration curves were used with each run and each curve was used to quantify half of the replicates for each sample. This precision protocol was run using both the manual and the automatic analyzers, and precision was evaluated separately for each method.

   Testing for the automated device was conducted using a single kit lot and one operator, and testing for the manual device was conducted using two kit lots, one instrument, and one operators. The results are summarized in the tables below:

Manual TGAL Precision

	Normal	Near Cutoff	Galactosemic
n (# of observations)	80	80	80
Mean (mg/dL)	3.5	10.0	31.3
Sr (within-run	0.291	0.640	1.401
C.V. (within-run)(%)	8.3	6.4	4.5
B (daily mean precision)	0.194	0.453	0.661
ST (total precision)	0.342	0.768	1.509
C.V. (total)(%)	9.8	7.7	4.8

SPOTCHECK TGAL Pro (automated) Precision

	Normal	Near Cutoff	Galactosemic
n (# of observations)	80	80	80
Mean (mg/dL)	3.3	10.2	33.1
S_r (within-run precision)	0.249	0.593	1.465
C.V. (within-run)(%)	7.5	5.8	4.4
B (daily mean precision)	0.122	0.324	0.937
S_T (total precision)	0.270	0.659	1.700
C.V. (total)(%)	8.2	6.5	5.1

b. Linearity/assay reportable range:

The claimed measuring range for the SPOTCHECK Neonatal Total Galactose Microplate Reagent Kit is 1.4 to 50 mg/dL for both the manual and SPOTCHECK Pro (i.e. automated) methods. This range is supported by the linearity and detection limits studies.

Linearity studies were performed as recommended in CLSI Guideline EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach.*

One human whole blood specimen with hematocrit adjusted to 55% was spiked with 50:50 mix of galactose/galactose-1-phosphate to provide 18 concentrations at equally spaced intervals. Four replicates at each level were analyzed in a single run using both manual and SPOTCHECK Pro processing.

To evaluate the data for linearity, first, second and third order regressions were performed to determine if any non-linear terms were significantly different from zero. Significance of the non-linear terms is determined through a t-test per CLSI EP6-A.

Fitted regression models for Manual method are:

Linear: $y = 0.955x - 0.318$
Second order: $y = 0.00x^2 + 0.959x - 0.362$
Third order: $y = 0.00x^3 - 0.003x^2 + 1.033x - 0.800$

Fitted regression models for SPOTCHECK Pro method are:

Linear: $y = 1.014x – 0.710$
Second order: $y = 0.00x^2 + 1.019x – 0.768$
Third order: $y = 0.00x^3 - 0.001x^2 + 1.036x – 0.871$

The t-test results for the non-linear terms of the second (b2) and third (b3) order regression analysis indicate that non-linear terms are not significant for both manual and SPOTCHECK Pro processing. Because there are no significant non-linear terms and the goal for repeatability has been met, the system is judged to be linear in the range of the LoQ to the high calibrant (1.4 to 50 mg/dL for manual and 1.4 to 50 mg/dL for SPOTCHECK Pro).

The package insert states that values below 1.4 mg/dL and above 50 mg/dL should be reported as <1.4 mg/dL or > 50 mg/dL.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The kit calibrators are traceable to a purchased certified reference galactose material. Each primary stock solution and new calibrator lot is tested and compared to the reference standard stock solution, and acceptability of less than 2% difference of each new lot when compared to the standard stock solution is required for lot release.

Real-time stability including shelf-life, transport, and in-use stability studies for the entire kit are performed, including the calibrators. Additionally, accelerated stability studies are also performed separately for the entire kit (including calibrators). The study protocols and statistically calculated acceptance limits were reviewed and found to be acceptable. Stability claims include 2 years storage unopened at 2 - 8ºC, and 28 days stored after reconstitution at 2 - 8ºC.

d. Detection limit:

The Limit of the Blank (LoB), Limit of Detection (LoD) Limit of Quantitation (LoQ) studies for the SPOTCHECK Neonatal Total Galactose Microplate Reagent Kit were conducted using CLSI Guideline EP 17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*.

Limit of the Blank was calculated for a total of 60 measurements of a blank control (blank filter paper punch). The LoB was determined separately on the manual TGAL operation and the SPOTCHECK Pro.

The LoB was estimated to be:

1.1 mg/dL (manual)

0.9 mg/dL (SPOTCHECK PRO)

The **Limit of Detection (LoD) and the Limit of Quantitation (LoQ)** were determined from low concentration (normal) samples prepared to simulate neonatal blood and spotted on filter paper and one kit lot, analyzed 30 times per plate on 2 separate plates for a total of 60 observations on each of 3 samples for both manual and SPOTCHECK Pro processing. Prior to spotting, human serum and human red blood cells are combined to approximate a representative neonate hematocrit of 55%. To establish the LoQ, since an estimate of bias is not assured, the following goal for Total Error (TE) was used: "imprecision at any concentration greater than or equal to the LoQ shall not exceed 20%". The LoD was estimated to be:

1.4 mg/dL (manual)

1.3 mg/dL (SPOTCHECK Pro)

Since the TE was less than the LoD, LoQ = LoD. Therefore the LoQ was estimated to be:

1.4 mg/dL (manual)

1.3 mg/dL (SPOTCHECK Pro)

However, the LoD for the kit was conservatively set as 1.4 mg/dL for both methods.

e. Analytical specificity:

Potential interference from several compounds was tested according to CLSI EP07-A2. Two levels of potential interferent and three levels of total galactose (i.e., normal, near cut-off, galactosemic) were evaluated. All samples were analyzed in a single run with 8 replicates from each control and test sample set. Significant interference was defined as a percent difference from the control sample of >10%. The tested compounds are listed in the following table:

Potential Interferent	**Highest tested concentration**
Albumin	6000 mg/dL
Ascorbate	6 mg/dL
Conjugated Bilirubin	20 mg/dL
Fructose	25 mg/dL
γ-Globulin	6000 mg/dL
Glucose	1200 mg/dL
Glutathione	60 mg/dL
Hemoglobin	200 mg/dL
Mannose	5 mg/dL
Sulfamethoxazole	4 mg/dL
Triglycerides	3264 mg/dL
Trimethoprim	4 mg/dL
Unconjugated bilirubin	20 mg/dL

The results, as summarized in the package insert, are:

"The following substances were tested and no evidence of interference found: conjugated bilirubin up to 20 mg/dL, unconjugated bilirubin up to 20 mg/dL, protein (albumin and gammaglobulin) up to 6000 mg/dL, hemoglobin up to 200 mg/dL, sulfamethoxazole up to 4 mg/dL, ascorbate up to 6 mg/dL, glucose up to 1200 mg/dL, mannose up to 5 mg/dL. There is evidence that lipemia with lipid concentrations of 3264 mg/dL, fructose concentrations of 25 mg/dL, glutathione concentrations of 60 mg/dL, or trimethoprim concentrations of 4 mg/dL may increase assay response slightly at low (normal) levels of total galactose, but that effect was not seen at a concentration near the projected cutoff or at elevated concentrations for any of the potential interferents, and is of no clinical significance."

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A total of 2037 specimens from newborns were obtained prospectively from a state public health laboratory's routine newborn screening program. Additionally, 51 manufactured blood spot specimens with elevated galactose were used in the method comparison study. Dried blood spots were assayed using the predicate device and results were compared with analysis results on the candidate device using both the manual and automated method.

Method Comparison Regression Analysis:

Predicate Device vs. SPOTCHECK TGAL Manual using n = 1166 (samples above and below the measuring range were not included in the analysis):

$Y = 0.911x – 0.146$

$R^2 = 0.982$

Predicate Device vs. SPOTCHECK TGAL Pro (automated) using n = 1092 (samples above and below the measuring range were not included in the regression analysis):

$Y = 0.946x – 0.156$

$R^2 = 0.982$

b. Matrix comparison:

Not applicable. The device is to be used only with neonatal whole blood.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):
The samples in the method comparison study (see above, M.2.a) were also analyzed to evaluate screening performance of the SPOTCHECK TGAL device. The presumptive positive cut-off values were established from the results using each kit's 99th and 99.5th percentiles:

TGAL	Predicate device	Proposed device	
		Manual	Automated
99th percentile	7.2 mg/dL	6.7 mg/dL	7.0 mg/dL
99.5th percentile	8.8 mg/dL	8.5 mg/dL	8.9 mg/dL

Screening Study 1: Screening Results Comparison for 2037 prospective samples and 51 manufactured samples:

SPOTCHECK TGAL Manual vs. Predicate Device:

Summary of Screening Agreement using the 99th percentile				
		Predicate Device		
		Positive	Negative	Totals
Candidate Device	Positive	89	2	91
	Negative	2	2175	2177
	Totals	91	2177	2268
Percent Positive Agreement = 97.8%				
Percent Negative Agreement = 99.9%				

Summary of Screening Agreement using the 99.5th percentile				
		Predicate Device		
		Positive	Negative	Totals
Candidate Device	Positive	76	3	32
	Negative	3	2186	2177
	Totals	79	2189	2209
Percent Positive Agreement = 96.2%				
Percent Negative Agreement = 99.9%				

SPOTCHECK TGAL Pro (automated) vs. Predicate Device

Summary of Screening Agreement using the 99th percentile				
		Predicate Device		
		Positive	Negative	Totals
Candidate Device	Positive	86	5	91
	Negative	5	2	2176
	Totals	91	2	2267
Percent Positive Agreement = 94.5%				
Percent Negative Agreement = 99.8%				

Summary of Screening Agreement using the 99.5th percentile				
		Predicate Device		
		Positive	Negative	Totals
Candidate Device	Positive	75	4	79
	Negative	4	2184	2188
	Totals	79	2188	2267
Percent Positive Agreement = 94.9%				
Percent Negative Agreement = 99.8%				

Screening Study 2: A supplemental study was performed to evaluate the screening performance of the SPOTCHECK TGAL assay with 11 retrospectively collected samples from newborns with diagnosed galactosemia along with 8 manufactured samples elevated in total galactose and 161 presumed negative neonatal specimens. The samples from newborns with Classic Galactosemia were analyzed randomly amongst the presumed negative neonatal specimens and manufactured samples.

SPOTCHECK TGAL Manual vs. Predicate Device:

Summary of Screening Agreement using the 99th percentile				
		Predicate Device		
		Positive	Negative	Totals
Candidate Device	Positive	19*	0	19
	Negative	1‡	160†	161
	Totals	20	160	180

* Nine samples screen positive with both the predicate and the SPOTCHECK TGAL Manual were from newborns confirmed to have Galactosemia. The other ten samples were the 8 manufactured samples and 2 samples from unaffected newborns (false positives).

Two samples that were GALT deficient but had normal total galactose screening during the initial screening performed in the laboratory had the following results:

‡ One sample from a newborn confirmed to have Galactosemia was screen positive with the predicate and screen negative with the SPOTCHECK TGAL Manual. This sample was later found to be from a baby with Classic Galactosemia who had been on soy formula since birth; a sample from a baby on soy formula is known to be a cause of false negative results (noted in the limitations of the assay).

† One sample from a newborn confirmed to have Galactosemia was screen negative with both the predicate and with the SPOTCHECK TGAL Manual.

The package insert states: "Samples from infants who are on soy formula or who have not received a milk feeding prior to sampling may provide a false negative result[1,2]. Samples from infants who have received a blood transfusion may also lead to a false negative result.[3]"

Summary of Screening Agreement using the 99.5th percentile			
	Predicate Device		
	Positive	Negative	Totals

[1] Kirkman HN. Newborn screening in North Carolina: the evolution of policy and practice, N C Med J., 69 (2), 92-7, 2008

[2] Kaye CI; Committee on Genetics, Accurso F, La Franchi S, Lane PA, Hope N, Sonya P, G Bradley S, Michele A LP. *Newborn screening fact sheets*, Pediatrics, 118 (3), 934-63, 2006

[3] Sokol, R.J., McCabe, E.R., Kotzer, A.M. and Langendoerfer, S.I., *Pitfalls in Diagnosing Galactosemia: False Negative Newborn Screening Following Red Blood Cell Transfusion*, J. Ped. Gastroent. Nutr., 8, 266-8, 1989

Candidate Device	Positive	17*	1[a]	18
	Negative	1[a]	161†	162
	Totals	18	162	180

* Nine samples screen positive with both the predicate and the SPOTCHECK TGAL Manual were from newborns confirmed to have Galactosemia. The other eight samples were the manufactured samples.

† Two samples that were GALT deficient but had normal total galactose screening during the initial screening performed in the laboratory were screen negative with both the predicate and with the SPOTCHECK TGAL Manual.

[a] There were two discrepant samples when measurements obtained with the proposed assay were compared to the predicate measurements. However, these samples were originally classified as screen negative sample therefore of the 180 samples tested in the supplemental study; two are false positives when the 99.5th percentile is applied.

SPOTCHECK TGAL Pro (automated) vs. Predicate Device

Summary of Screening Agreement using the 99th percentile				
		Predicate Device		
		Positive	Negative	Totals
Candidate Device	Positive	20*	0	20
	Negative	0	160†	160
	Totals	20	160	180

* Ten samples screen positive with both the predicate and the SPOTCHECK TGAL Pro (automated) were from newborns confirmed to have Galactosemia. The other ten samples the 8 manufactured samples and 2 samples from unaffected newborns (false positives).

† One sample that was GALT deficient but had normal total galactose screening during the initial screening performed in the laboratory was screen negative with both the predicate and with the SPOTCHECK TGAL Pro (automated).

Summary of Screening Agreement using the 99.5th percentile				
		Predicate Device		
		Positive	Negative	Totals
Candidate Device	Positive	17*	1[a]	18
	Negative	1[a]	161†	162
	Totals	18	162	180

* Nine samples screen positive with both the predicate and the SPOTCHECK TGAL Pro (automated) were from newborns confirmed to have Galactosemia. The other eight samples were the manufactured samples.

† Two samples that were GALT deficient but had normal total galactose screening during the initial screening performed in the laboratory were screen negative with both the predicate and with the SPOTCHECK TGAL Pro (automated).

[a] There were two discrepant samples when measurements obtained with the proposed assay were compared to the predicate measurements. However, these samples were originally classified as screen negative sample therefore of the 180 samples tested in the supplemental study; two are false positives when the 99.5th percentile is applied.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

The summary of the results from the 2037 newborn specimens and 51 manufactured blood spot specimens tested with this kit is shown in the table below:

	SPOTCHECK Manual	**SPOTCHECK Pro Automated**
99.5th percentile	8.5 mg/dL	8.9 mg/dL

The package insert includes precautionary language that each laboratory should establish their own reference range and cut-off values and that cut-offs from another galactose screening test should not be used. Also included in the labeling is the recommendation that screening presumptive positive samples be tested with a confirmatory diagnostic method and to follow local requirements for follow up testing.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.